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TECHNICAL NOTE

Successful production of sterile pyrogen-free electrolyte solution by ultrafiltration

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Sterile pyrogen-free solutions in large quantity are required for medical procedures such as peritoneal dialysis (50 to 100 liters/treatment given i.p.) and hemofiltration, a new potentially useful process of convective blood cleansing (40 to 70 liters/treatment given i.v.). These solutions contribute significantly to the overall cost of these procedures. In an effort to reduce this cost, the following method of solution production was developed and tested.

Methods

Water treated for routine use in artificial kidney dialysis fluid was utilized (treated by reverse osmosis with a final "polish" using a deionizer). A resistivity of at least 1 megohm-cm is a commonly used criterion of satisfactory quality [1]. Water processed in this manner when tested with limulus lysate (supplied by DIFCO, Cat. #3361-59) and culture, however, showed the presence of both pyrogen and small numbers of gram-negative bacteria. Growth of bacteria on the downstream side of the reverse osmosis membrane system and, more commonly, in the resin beds of the deionizer has been reported by others [2] and must account for their presence in the product water. Processed water was combined with a single concentrate of electrolytes and glucose (supplied by Erika Co., Cat. #08-8110-2) to achieve the following concentrations: sodium, 140 mEq/liter; chloride, 106 mEq/liter; acetate, 41 mEq/liter; potassium, 2.0 mEq/liter; calcium, 3.5 mEq/liter; magnesium, 1.5 mEq/liter; and glucose, 100 mg/100 ml.

Forty-liter batches were prepared in glass carboys equipped with stainless steel caps which contained a cotton-plugged air vent and a dip tube through which to retrieve the solution. Quality control of the solution composition was then carried out. In this instance, osmolality and conductivity were measured to assure appropriate dilution of the concentrate. Next, using the flow circuit shown in Fig. 1, a 1.6 m^2 hollow-fiber ultrafilter made of an anisotropic polyelectrolyte membrane (XP-50 1.6 m^2 hollow-fiber ultrafilter for biological use, supplied by Amicon Corporation, Lexington, Mass.) was used to remove pyrogens and bacteria. Each ultrafilter was initially qualified for possible "pin hole" leaks or broken fibers by ultrafiltering blue dextran (2×10^6 daltons) solution and visually examining the ultrafiltrate for color. The assembled ultrafilter was checked to be free of pyrogen by using the limulus lysate assay on saline solution left standing in the fiber lumen and in the casing. Removal of the pyrogens and bacteria from the electrolyte solution was accomplished by ultrafiltration (500 to 600 mm Hg driving pressure) into a second 40-liter glass carboy that had been heat sterilized and depyrogenized (200° C for 12 hr). Sterility of the produced solution was checked with routine aerobic and anaerobic cultures taken through the dip tube at the top of the carboy, and pyrogen was checked for with the limulus lysate assay. Bacterial pyrogen deliberately introduced upstream of the membrane was totally excluded from the product solution, indicating a "cutoff" for the XP-50 ultrafilter that satisfactorily blocks passage of pyrogen and bacteria. The system was filled with formalin when not in use. The sterilant was introduced through the ultrafilter in a manner entirely analogous to that used to prepare the sterile pyrogen-free solution. This preserved the pyrogen-free status of that portion of the system downstream of the filter.

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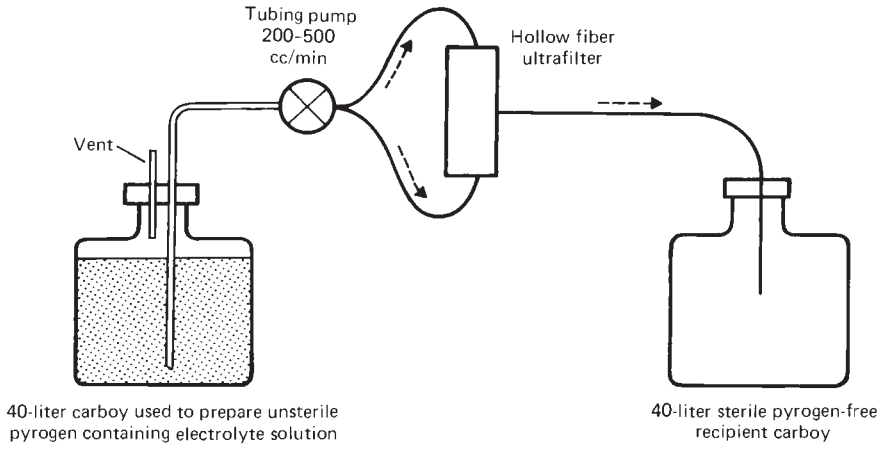

Fig. 1. *Diagrammatic sketch of membrane depyrogenation process.*

Results

Solution qualified as free of bacteria and negative to limulus lysate was introduced as diluting fluid into the blood path of an extracorporeal circuit used for hemofiltration at a rate of 200 cc/min (Fig. 2). The same kind of ultrafilter used to depyrogenize the solution originally was then used to remove the excess fluid so that volunteer patient blood volume was not altered. Thirty to fifty liters of this diluting fluid was delivered into the blood path over the course of the 4- to 5-hr period of hemofiltration treatment. To date, more than 8,000 liters of solution prepared in this manner have been used in seven patient volunteers with no untoward responses.

Two 40-liter carboys prepared in this manner and sampled at 10 weeks were sterile and pyrogen-free. No formal study of filter life was undertaken; rather, ultrafilters used to prepare the solution were discarded after preparation of 500 to 800 liters. There is no reason to expect that filter life could not

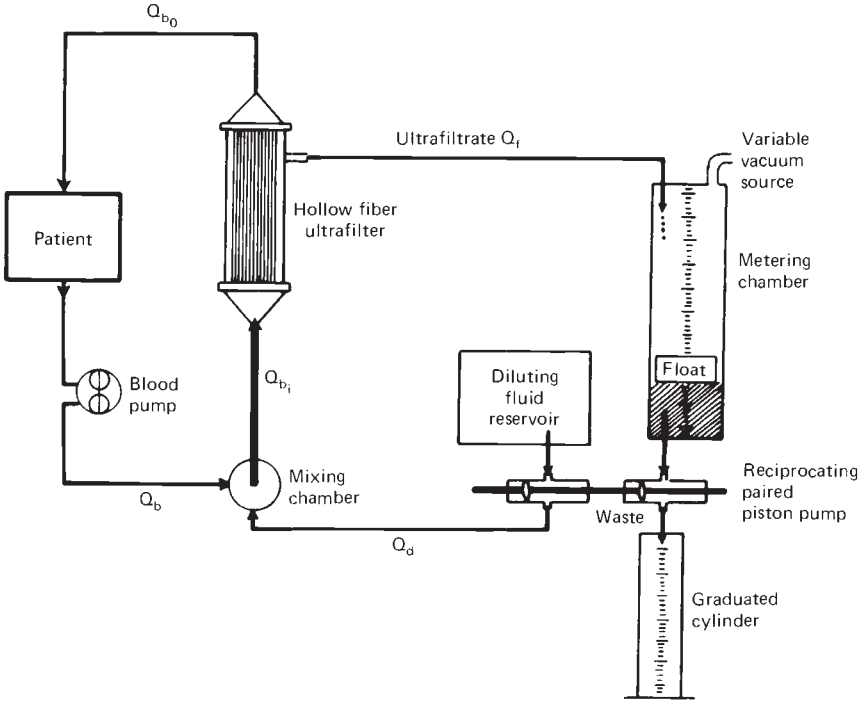

Fig. 2. *Diagrammatic representation of hemofiltration.* This shows a process whereby whole blood leaving the patient at a flow rate Q_b is diluted with saline solution at a flow rate of Q_d and is then ultrafiltered at a rate Q_f, such that Q_f just matches Q_d.

be prolonged easily by back-flushing the membrane to wash away the accumulated debris.

The cost of solution prepared in this manner, including aerobic and anaerobic culture, limulus lysate assay, cost of the XP-50 ultrafilter, and personnel time was less than a dollar ($0.90/liter) as contrasted with over two dollars ($2.50/liter) for commercially supplied solution purchased direct from the supplier with no intermediary markup.

Discussion

Pyrogen in water solution is generally considered to be of large molecular weight (i.e., in the millions) with smaller molecular weight fragments being nontoxic [3–5]. The XP-50 membrane passes inulin (5,200 daltons) completely, but rejects (99+%) albumin (60,000 daltons) [6]. The limulus lysate assay is reported to be sensitive to the presence of gram-negative endotoxin at a concentration of 10^{-9} to 10^{-14} μg/ml [7].[a] Presence of endotoxin at concentrations below the sensitivity of the method might be expected to show up clinically as a pyrogen response if the large volumes of solution utilized brought the total delivery of endotoxin to a toxic level in the blood stream. No such event was noted for volumes of up to 40 liters delivered in a 5-hr period.

The limulus lysate test determines the presence of gram-negative bacterial products and would not be expected to show the presence of pyrogens such as colloidal iron, silica, clay, etc. Reverse osmosis treatment of the water would remove such contaminants, however, and their presence in dialysis fluid concentrate where substantial concern over trace presence of any extraneous substances is high would be unlikely. Rabbit assay for pyrogen which would be expected to reveal gram-negative and other nonbacterial pyrogens, although not used routinely, has proved to be negative on spot check. In like manner, the growth of gram-positive organisms in the water processing system as a result of retrograde contamination of the reverse osmosis membrane or the deionizer resin beds after removal of the chlorine by the latter is equally unlikely in view of their more demanding growth requirements. In addition, should this event occur, one might reasonably expect that both gram-positive bacteria and their wall fragments would be of a molecular size comparable to their gram-negative counterparts and hence be removed by the ultrafilter. Clearly, starting water should not contain trace elements and/or undesirable substances below the molecular weight cutoff of the ultrafilter. Similarly, additives such as the electrolyte concentrate should be carefully examined to assure satisfactory purity.

The cost of the ultrafilter at $125 each is high because they are made in limited numbers for supply to an experimental protocol. Cost of these items is highly labor intensive and could be expected to fall substantially if large numbers are produced. Further, the units can be back-flushed when flux rate slows, and continued performance at 200 cm^3/min of product solution can be expected for use beyond the 800 liter cutoff presently exercised. Ultrafilter life may be further prolonged by adding a 0.2-μ filter proximal to the ultrafilter to trap bacteria and other particulates that may be present.

Preparation of sterile pyrogen-free peritoneal dialysis solution with the process of reverse osmosis requires addition of sterile pyrogen-free electrolyte concentrate to the processed water and, as such, is inherently a more expensive process than that described. Further, the driving pressure and/or membrane area required to achieve satisfactory flow rates for water in a reverse osmosis system are substantially larger than that required for the "looser" XP-50 ultrafiltration membrane, making the supporting equipment necessary to operate the latter system a good deal simpler.

There are several applications for this one-step low-pressure filtration process for making biologically safe electrolyte solution. Peritoneal dialysis has been roughly comparable in expense to hemodialysis, due in large measure to the cost of purchasing the dialysis solution. The work of Tenckhoff, Meston, and Shilipetar [9] to produce this solution "on line" by using reverse osmosis to treat tap water and by subsequently adding sterile pyrogen-free electrolyte concentrate has done much to reduce the cost of this technique. The present method would reduce cost even more by obviating the need for concentrate to be sterile and pyrogen-free. If municipal tap water is of satisfactory quality from the standpoint of trace elements, no prior water treatment may be necessary. The ASAIO and AAMI Kidney Standards Subcommittee has suggested that tap water used in preparation of dialysis fluid should meet the requirements for purified water as described in the U.S. Pharmacopeia [10] with the following additions: *(1)* The heavy metal content of water shall not exceed 0.1 mg/liter. *(2)* The arsenic

[a] It should be noted that the limulus amebocyte lysate assay for pyrogen has now been licensed by the Food and Drug Administration as an alternative for the rabbit pyrogen test for detecting pyrogens in biological products and medical devices [8].

content shall not exceed 0.1 mg/liter. *(3)* The total viable microbial count shall not exceed 100/ml. *(4)* If water to be used is treated with a deionizer, then a resistivity of the product should be greater than 1×10^6 ohm-cm at 20° C. Requirement 3 would not apply for water used in the present process.

Hemofiltration is another area for application. At present this technique for blood cleansing must be considered experimental. Reports from our laboratory and others in Europe [11–14] on improved correction of the uremic syndrome make it likely that hemofiltration which requires 30 to 70 liters of i.v. saline solution per treatment will find low pressure filtration useful to reduce treatment costs. Other potential applications may only be speculated on.

It should be noted that although there are requirements in the form of good manufacturing practices presented by the Food and Drug Administration for solutions that are transported across state lines, it is left to individual institutions (hospitals, pharmacies) to control the quality of extemporaneously compounded solutions as presently described. Further, there is nothing at present in the pending Food and Drug Administration devise legislation that specifically addresses ultrafiltration membranes used in the manner described.

Last, the logical next step for procedures such as peritoneal dialysis and hemofiltration is to prepare solution by ultrafiltration for use "on line," provided suitable quality control may be maintained. For example, two ultrafiltration membranes in series with a sampling port for serial pyrogen-testing between the two units may be used to insure that a broken fiber occurring in an "on line" system does not jeopardize the safety of the patient.

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